**Additional file 3-Supplementary Materials and Methods**

**Animals**

Male 615 mice (4-6 weeks old) were housed in specific pathogen-free conditions. All animals were fed a standard diet ad libitum and housed in a temperature-controlled animal facility with a 12/12-hour light/dark cycle. All animals received humane care according to criteria outlined in the “Guide for the Care and Use of Laboratory Animals” (NIH publication 86-23 revised 1985).

**Cell lines**

The GC cell lines were purchased from the cell bank of the Chinese Academy of Sciences (Shanghai, China). The mouse GC cell line, MFC cells, was maintained in RPMI-1640 (Gibco BRL, MD, USA) supplemented with 10% (v/v) fetal bovine serum (FBS) (Gibco BRL, MD, USA) at 37°C in a humidified incubator containing 5% CO2. The human GC cell lines, MKN45 and N87 cells, were routinely maintained in Dulbecco’s modified Eagle’s medium (DMEM) (Gibco BRL, MD, USA) supplemented with 10% (v/v) fetal bovine serum (FBS) (Gibco BRL, MD, USA) at 37°C in a humidified incubator containing 5% CO2.

**CCK-8 assays**

The cell proliferation was assessed with the cell counting kit-8 (Dojindo, Kumamoto, Japan). Briefly, GC cells (4-5 × 103 per well) were plated in 96-well plates in RPMI-1640 or DMEM supplemented with 10% FBS and incubated at 37°C, 5% CO2. After 12 hours, indicated concentrations of the compounds were added into the wells, and the cells were incubated for another 24-72 hours. The cells were then incubated for an additional 2 hours with CCK-8 reagent (100μl/mL medium) and read at 450 nm using a microplate reader (Thermo, MD, USA). Each experiment was reproduced in six wells and repeated at least three times.

**Enzyme-Linked Immunosorbent Assay (ELISA)**

Blood samples from mice were subjected to centrifugation at 2000 rpm for 10 minutes at 4°C. The supernatant plasma was then collected. The serum was centrifuged at 12,000 rpm for 15 minutes at 4°C to remove cell debris and fractioned into multiple aliquots for storage at -80°C. The IFN-γ(MIF00), TNF-α (MTA00B), IL-2 (M2000) and IL-10 (M1000B) ELISA kits were purchased from R&D Systems (MN, USA). The experiments were performed according to the manufacturer’s instructions. All the data presented were normalized to the total amounts of protein present in the extracts. Briefly, 100μL of sample was added to each well and incubated the plates for 2.5 hours at RT. The plates were washed and incubated with the conjugate for 2 hours. After washing, substrate solution was added to determine immunoreactivity, and the absorbance was determined using a Microplate Spectrophotometer (Bio-Rad). A curve of the absorbance versus the concentrations of cytokines/chemokines in the standard wells was plotted.

**Immunohistochemical analysis, immunofluorescence staining and evaluation of imunohistochemical variables**

Harvested tumors were fixed in 4% formaldehyde solution in PBS, and embedded in paraffin. 5mm thick sections were cut from paraffin-embedded tissue blocks, deparaffinized and rehydrated in ethanol, and then subjected to antigen retrieval. Endogenous peroxidase activity was blocked using 0.3% hydrogen peroxide in methanol for 30 minutes. Sections were blocked with 2% BSA in PBS for 1hour at 37 °C, followed by incubation with primary antibodies at 4°C overnight. After washing with PBS three times on the second day, corresponding secondary antibodies were applied, and samples were further incubated at 37 °C for 1 hour. Slides were visualized with DAB staining. For immunofluorescence staining, the sections were incubated with the primary antibodies overnight at 4 °C. Then, samples were incubated with FITC conjugated and Tex-Red-conjugated secondary antibodies for 2 hours at 37 °C. Immunohistochemical staining and immunofluorescence staining were assessed by 3 independent investigators who were blinded to tumors characteristics, and discrepancies were resolved by consensus. Photographs of five representative fields were captured under high-power magnification (400×/200×) by the Leica QWin Plus v3 software; identical settings were used for each photograph. Negative controls were obtained by omitting the primary antibody. For analyzing PD-L1 expression, the staining intensity was scored as 0=no staining, 1=weak staining, 2=moderate staining, and 3=strong staining; the percentage of positively stained cells were scored as 0=no staining, 1=0-25% staining, 2=25%-50% staining, 3=50%-75% staining and 4= 75%-100% staining; the final IHC score was calculated by multiplying the intensity score with the percentage score. Quantification of CD31 and MECA-79 staining in tumor tissues of xenografts was calculated as the number of CD34-positive vessels/field or MECA-79-positive vessels /field. For quantification of Ki-67 and HIF-1α staining, only nuclear immunoreactivity was considered positive; the Ki-67 and HIF-1α score were corresponded to the number of labeled Ki-67 or HIF-1α cells among at least 500 cells per region and were expressed as percentages. Quantification of CD8+T cells, CD4+ T cells, CD11c+ DC cells, DC-LAMP(LAMP3) and CD20+B in tumor tissues of xenografts was calculated as the number of CD8+T cells, CD4+ T cells, CD11c+ DC cells, DC-LAMP(LAMP3) and CD20+B /field.

**Xenograft tumor models**

To investigate the efficacy of dual apatinib/aPD-L1 treatment in mouse GC models, MFC cells (5×106 cells in 100 μL of PBS) were subcutaneously injected into the right flank region of 615 mice. Once tumors were palpable, the mice were then randomly assigned into 4 groups: (1) solvent control (ddH2O, orally); (2) anti-PD-L1 antibody (aPD-L1, 10mg/kg/tiw, i.p.); (3) Apatinib (150 mg/kg/d, orally); (4) combined apatinib/aPD-L1 (apatinib,150 mg/kg/d, orally + aPD-L1, 10mg/kg/tiw, i.p.). The survival was recorded daily until a time-defined endpoint.

To investigate whether the combination of apatinib and anti-PD-L1 antibody had a synergistic impact on survival, we calculated the expected additive curve as described by Lydia Meder and colleagues in recent published paper (Meder L et al. 2018). Let*P*A (t), *P*B (t), *P*A,B (t) and *P*Cntrl(t) denote the probability of survival for t∈ [0; ∞) under therapy with compound 𝐴, 𝐵, their combination or vehicle solution, respectively. The expected additive curve was calculated as

*P*𝐴+𝐵 (t):=min(1, *P*Cntrl(t) + |*P*A (t) - *P*Cntrl(t)|≥0 + |*P*B (t) - *P*Cntrl(t)|≥0)

where *P*A+B (t) denotes the expected probability of survival, assuming the combination effect of drugs A and B is additive, and |a|≥0 :=max (0, a). The expected number of events at each time point of *P*A+B (t) was calculated by inverting the Kaplan-Meier statistics, assuming equal cohort sizes between combination and monotherapy cohorts. Finally, we compared the expected additive survival rate *P*A+B (t) with the observed survival rate *P*A,B (t) under drugs 𝐴 and 𝐵 in combination, using a Mantel-Cox test.

To investigate the role of LTβR signaling in the efficacy of dual apatinib/aPD-L1 treatment in mouse GC models, MFC cells (5×106 cells in 100 μL of PBS) were subcutaneously injected into the right flank region of 615 mice. Once tumors were palpable, the mice were then randomly assigned into 4 groups: (1) solvent control (ddH2O, orally); (2) LTβR antagonist (2mg/kg/tiw, i.p.); (3) combined apatinib/aPD-L1 (apatinib,150 mg/kg/d, orally+ aPD-L1, 20mg/kg/tiw, i.p.); (4) combined apatinib/aPD-L1/ LTβR antagonist (apatinib,150 mg/kg/d, orally + aPD-L1,10mg/kg/tiw, i.p. + LTβR antagonist,2mg/kg/tiw, i.p.) .

**References:**

Meder L, Schuldt P, Thelen M et al (2018) Combined VEGF and PD-L1 Blockade Displays Synergistic Treatment Effects in an Autochthonous Mouse Model of Small Cell Lung Cancer. Cancer Res 78: 4270-4281. https ://doi.org/10.1158/0008-5472.CAN-17-2176